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ON THE ACTIVATION OF OPSONIN IN HEATED HUMAN SERUM.*

MODIFICATION OF WRIGHT'S HEATED SERUM TEST FOR TUBERCULOSIS.

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WITH the idea of showing whether any relation could be demonstrated between the thermostabile (found in infected cases) component of opsonin and that of complement, some experiments were undertaken which have at this stage resolved themselves into what might be termed a modification of Wright's Heated Serum Test.

Wright's Heated Serum Test is briefly as follows: Both the immune and normal sera are heated for 10 minutes at 60° C. and the phagocytic index taken. It will usually be found that the sera from cases infected with tuberculosis retain their phagocytic power in marked contrast to the heated normal sera whose indices are usually nothing or at most found in the second decimal place (as 0.05). Thus there is induced in the serum of the patient undergoing auto-inoculation a thermostabile substance capable of producing phagocytosis. Occasionally this substance is not demonstrable in undoubted cases. This loss of phagocytosis was investigated by adding a fourth volume of complement (in the form of fresh diluted rabbit serum). Under these conditions the sera from infected cases gave a diagnostic phagocytosis while the loss of phagocytosis of normal sera was unaffected. This is shown in the following experiments in which the serum used was heated to 60° C. for 10 minutes, the complement being rabbit serum diluted 1:12.

EXPERIMENT 1.

	Phagocytic Index of Heated Serum Alone	Phagocytic Index of Heated Serum + Complement	Phagocytic Index of Heated Serum + Salt (1.5%)
Patient A.....	0.05	0.28
" B.....	0.02	0.22	0.03
" C.....	0.42
Normal a.....	0.00	0.01	0.04
" b.....	0.04	0.05
R. S. 1½.....	0.04	0.04

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EXPERIMENT 2.

	Phagocytic Index of Heated Serum Alone	Phagocytic Index of Heated Serum + Complement
Patient D.....	0.02	0.18
" E.....	0.21	0.21
" F.....	0.04	0.03
Normal a.....	0.03	0.03
" b.....	0.03	0.05
R. S. $\frac{1}{12}$	0.01

EXPERIMENT 3.

(In this the sera were heated at 56° for 30 minutes.)

	Phagocytic Index of Heated Serum + Complement
Patient G.....	0.28
" H.....	0.09
" I.....	0.17
" J.....	0.40
" K.....	0.18
" L.....	0.01
Normal a.....	0.05
" b.....	0.05
R. S. $\frac{1}{10}$ + salt (1.5 %).....	0.07

EXPERIMENT 4.

The sera were heated for 20' at 60° on the same set as in Experiment 5. This exposure destroyed the phagocytosis in all sera, no count being more than 0.04 as given by the diluted rabbit serum.

EXPERIMENT 5.

	Phagocytic Index of Serum Alone	Phagocytic Index of Serum + Com- plement
Patient M.....	0.14
Normal a.....	0.02
" c.....	0.02
R. S. $\frac{1}{10}$	0.02

In all the experiments especial care was taken in the preparation of the tubercle emulsion (dried bacilli being ground up with 1.5 per cent salt solution and allowed to sediment in long tubes. The resulting emulsion happened to be free from clumps and gave a normal count of about 1.30 in a 20-minute incubation period. The same emulsion was used in all tests.

Several of the results in the experiments given have been confirmed with the same sera two or more times. In one instance a contradictory count was obtained. This has not been accounted for.

The clinical diagnosis in the cases utilized in testing for tuberculosis is as follows:

Patient A.—Pulmonary tuberculosis which at the time of the test showed a slight rise in temperature.

Patient B.—Advanced case of pulmonary tuberculosis.

Patient C.—Pulmonary tuberculosis which was at the time doing well and showed normal temperature.

Patient D.—Periorbital abscess, suspicious Calmette reaction.

Patient E.—Early pulmonary tuberculosis, positive Calmette reaction.

Patient F.—Lobar pneumonia.

Patient G.—Interstitial keratitis, positive Calmette reaction.

Patient H.—Rhinitis and laryngitis, negative Calmette test.

Patient I.—Tuberculous hip disease, positive Calmette reaction.

Patient J.—Tuberculous hip and double psoas abscess, positive Calmette reaction.

Patient K.—Cervical adenitis, with sloughing, negative Calmette test.

Patient L.—Anemia, negative Calmette test.

Patient M.—Tuberculosis of knee of two years' duration without much change.

Among these cases, Patient D shows a positive count of 0.18 and a suspicious Calmette reaction; Patient K, a negative Calmette and a positive count. Patient L, diagnosed as tuberculosis is the lowest count, being 0.14 with the R. S. 0.01; 0.05 is the highest count obtained from a normal.

In view of the fact that the detection of the specific amboceptor (as utilized by Wassermann, Plaut and others) is of slighter diagnostic value in tuberculosis than in syphilis, pertussis, gonorrhea, etc., some experiments were undertaken on four cases of gonorrheal arthritis, available at the time.

EXPERIMENT 6.

	Phagocytic Index of Heated Serum + Complement	Phagocytic Index of Heated Serum + Salt Solution
Patient A.....	2.06	1.90
" B.....	3.36
Normal a.....	0.80	0.76
" b.....	0.27

EXPERIMENT 7.

	Phagocytic Index of Heated Serum + Complement
Patient A.....	5.78
" C.....	5.00
Normal a.....	2.46
" b.....	2.82
R. S. $\frac{1}{8}$	1.94

EXPERIMENT 8.

	Phagocytic Index of Heated Serum + Complement
Patient D.....	1.28
Normal a.....	0.73
" b.....	0.68
R. S. $\frac{1}{16}$	0.74
Unheated Serum of Nor. A....	2.00

In the estimation of the index to the gonococcus emulsion a five to six hours' growth incubated for seven to eight minutes was employed.

Of the four cases of gonorrheal arthritis utilized two (A and B) were at the time being treated with killed gonococci, the remaining two (C and D) were under ordinary treatment without inoculation. Patients A, B, and C were diagnosed clinically as gonorrheal arthritis before the test; the fourth case (Patient D) has subsequent to the laboratory diagnosis been accepted as gonorrheal in origin.

In the results given no attempt has been made at completeness. They have to a considerable extent been obtained in a series which (not yet complete) was undertaken to show the specificity of opsonin to be closely bound up with the thermostable part, and to adduce experimental evidence supporting a treatment of bacterial diseases with both Wright's vaccines and serum.